

FORM PTO 1390
(REV 5-93)

US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NUMBER
2002-0279ATRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. §371U.S. APPLICATION NO.
(if known, see 37 CFR 1.52)
NEW

107089796

International Application No.
PCT/JP99/05758International Filing Date
October 19, 1999Priority Date Claimed
None

Title of Invention

BACTERIA GROWTH CARRIER INCLUDING TRACE ELEMENT

Applicant(s) For DO/EO/US

Takaaki MAEKAWA

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. §371(c)(2)). **ATTACHMENT A**
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3)).
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19.
9. ☒ An unexecuted oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)). **ATTACHMENT B**
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 14. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98. **ATTACHMENT C**
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A **FIRST** preliminary amendment.
 - ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☒ Other items or information:
 - ☒ a. Cover Page of Published International Application No. WO 01/29203 - **ATTACHMENT D**
 - ☒ b. International Search Report - **ATTACHMENT E**

U.S. APPLICATION NO. **10/069796**
NEWINTERNATIONAL APPLICATION NO.
PCT/JP99/05758ATTORNEY'S DOCKET NO.
2002-0279A

15. [X] The following fees are submitted

CALCULATIONS

PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee nor international search fee paid to USPTO
and International Search Report not prepared by the EPO or JPO \$1040.00
International Search Report has been prepared by the EPO or JPO \$ 890.00
International preliminary examination fee not paid to USPTO but international search
paid to USPTO \$ 740.00
International preliminary examination fee paid to USPTO but claims did not satisfy provisions
of PCT Article 33(1)-(4) \$ 690.00
International preliminary examination fee paid to USPTO and all claims satisfied provisions of
PCT Article 33(1)-(4) \$ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$890.00

Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest
claimed priority date (37 CFR 1.492(e)).

\$

Claims	Number Filed	Number Extra	Rate		
Total Claims	3 -20 =		X \$18.00	\$	
Independent Claims	1 - 3 =		X \$84.00	\$	
Multiple dependent claim(s) (if applicable)			+ \$280.00	\$	

TOTAL OF ABOVE CALCULATIONS =

\$890.00

[] Small Entity Status is hereby asserted. Above fees are reduced by 1/2.

\$

SUBTOTAL =

\$890.00

Processing fee of \$130.00 for furnishing the English translation later than [] 20 [] 30 months from the
earliest claimed priority date (37 CFR 1.492(f)).

+

\$

TOTAL NATIONAL FEE =

\$890.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an
appropriate cover sheet (37 CFR 3.28, 3.31). \$40 per property +

\$

TOTAL FEES ENCLOSED =

\$890.00

Amount to be refunded

\$

Amount to be charged

\$

a. [X] A check in the amount of \$890.00 to cover the above fees is enclosed. A duplicate copy of this form is enclosed.

b. [] Please charge my Deposit Account No. 23-0975 in the amount of \$_____ to cover the above fees.
A duplicate copy of this sheet is enclosed.c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 23-0975.**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b))
must be filed and granted to restore the application to pending status.

19. CORRESPONDENCE ADDRESS



000513

PATENT TRADEMARK OFFICE

By: _____

Matthew Jacob,
Registration No. 25,154WENDEROTH, LIND & PONACK, L.L.P.
2033 "K" Street, N.W., Suite 800
Washington, D.C. 20006-1021
Phone: (202) 721-8200
Fax: (202) 721-8250

February 28, 2002

THE COMMISSIONER IS AUTHORIZED
TO CHARGE ANY DEFICIENCY IN THE
FEE FOR THIS PAPER TO DEPOSIT
ACCOUNT NO. 23-0975.

[CHECK NO. 49068]

[2002-0279A]

(12)特許協力条約に基づいて公開された国際出願

(19) 世界知的所有権機関
国際事務局(43) 国際公開日
2001年4月26日 (26.04.2001)

PCT

(10) 国際公開番号
WO 01/29203 A1

- (51) 国際特許分類: C12N 11/08, (MAEKAWA, Takaaki) [JP/JP]; 〒305-0006 茨城県つくば市天王台1-1-1 Ibaraki (JP).
11/10, 11/14, C12M 1/40, C02F 3/10
- (21) 国際出願番号: PCT/JP99/05758 (74) 代理人: 弁理士 西澤利夫(NISHIZAWA, Toshio); 〒150-0042 東京都渋谷区宇田川町37-10 麻仁ビル6階 Tokyo (JP).
- (22) 国際出願日: 1999年10月19日 (19.10.1999)
- (25) 国際出願の言語: 日本語 (81) 指定国 (国内): CN, US.
- (26) 国際公開の言語: 日本語 (84) 指定国 (広域): ヨーロッパ特許 (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
- (71) 出願人 (米国を除く全ての指定国について): 科学技術振興事業団 (JAPAN SCIENCE AND TECHNOLOGY CORPORATION) [JP/JP]; 〒332-0012 埼玉県川口市本町4丁目1番8号 Saitama (JP).
添付公開書類:
— 国際調査報告書
- (72) 発明者; および
(75) 発明者/出願人 (米国についてのみ): 前川孝昭
- 2文字コード及び他の略語については、定期発行される各PCTガゼットの巻頭に掲載されている「コードと略語のガイダンスノート」を参照。

(54) Title: TRACE ELEMENT-CONTAINING CARRIER FOR GROWING MICROORGANISM

(54) 発明の名称: 微量要素を抱括した菌体増殖用担体

(57) Abstract: A carrier for culturing a microorganism wherein a polymer (11) containing trace elements and inorganic nutritious salts which are needed in the growth of the microorganism is sandwiched and laminated between inorganic porous materials (12). This carrier is useful in bioreactors having a high activity and a high microbial cell density, processing waste water, etc.

(57) 要約:

菌の増殖のための微量要素や無機栄養塩を抱括した高分子体(11)が無機質多孔体(12)により挟持積層されている菌体培養担体とし、高い活性と高密度菌体によるバイオリアクタや廃水処理等に有用な培養担体を提供する。

ATTACHMENT D

RECEIVED TIME FEB. 21. 4:12AM

20020221 05260001

WO 01/29203 A1

BACTERIA GROWTH CARRIER INCLUDING TRACE ELEMENT

FIELD OF THE INVENTION

The present invention relates to a trace element/inorganic nutrient salt diffusion-type bacteria culture carrier. More specifically, the invention relates to a trace element/inorganic nutrient salt diffusion-type bacteria culture carrier which is useful in a wastewater disposal apparatus, a food-making industry and a drug-producing industry.

BACKGROUND OF THE INVENTION

As a method for producing a carrier, a method in which bacteria or enzymes are included in a polymer gel (inclusion method) has been so far known, and it has been industrially utilized.

However, the ordinary method depends on a phenomenon that trace metal elements or inorganic nutrient salts useful for growth of bacteria are diffused and moved from an external culture solution to the inside of a carrier. Accordingly, a diffusion rate of these materials is controlled by the growth of bacteria. Further, since a metabolized substance has resistance to diffusion onto a surface of a carrier, which sometimes hinder the growth of bacteria. Still further, when a gaseous substance is metabolized, floating or destruction

of a carrier occurs. Furthermore, in the ordinary method such as the inclusion method, the activity of bacteria is notably decreased owing to toxicity of a polymer used. Therefore, even when a density of bacteria is increased, the activity thereof is not necessarily proportional to the density of bacteria. These are the problems of the ordinary method.

In order to solve these problems, a surface-binding-type carrier was developed in which bacteria and the carrier are physicochemically adhered.

Nevertheless, since this method depends on the physicochemical adhesion between a viscous polymeric substance secreted in the growth of bacteria and the carrier, the growth of bacteria is controlled by the composition of inorganic nutrient salts or trace elements of a liquid entered from an external solution. Moreover, it involves problems that when bacteria present on a surface of a carrier flow within a bioreactor, peeling of bacteria occurs and high-density enrichment culture is naturally restricted.

SUMMARY OF THE INVENTION

Under these circumstances, the invention aims to provide a novel trace element/inorganic nutrient salt diffusion-type bacteria culture carrier in which a high activity and a high density of bacteria can be realized in a bioreactor or a wastewater disposal apparatus.

The invention is to provide, upon solving the problems, a bacteria growth carrier including a trace element in which an element polymer product obtained by including a trace element for growth of bacteria in a synthetic or natural polymeric material is laminated by being held with a synthetic or natural inorganic porous material.

Further, the invention is to provide the bacteria growth carrier which is a particulate, cylindrical or plate-like carrier, or the bacteria growth carrier which has a honeycomb structure.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view showing a cylindrical carrier of the invention.

Fig. 2 is a perspective view showing a multiple cylindrical carrier.

Fig. 3 is a perspective view showing a flat-plate-like carrier.

Fig. 4 is a perspective view showing a part of a carrier of honeycomb structure.

Fig. 5 is a sectional view showing an example in which the carrier of the invention is installed in a bioreactor.

Figs. 6A and 6B are perspective views showing carriers in which plural holes are formed in rectangular and cylindrical porous materials and polymer products are packed therein.

Figs. 7A and 7B are a side sectional view and a plan view each showing an example in which carriers are installed in a bioreactor.

Fig. 8 is a sectional view showing an example in which carriers are installed in parallel to a flow within a bioreactor.

Figs. 9 is a sectional view showing an example in which carriers in Fig. 3, 4 or 6A are installed.

DETAILED DESCRIPTION OF THE INVENTION

In the invention, as the binding-type carrier, a useful substance such as a trace metal element or an inorganic nutrient salt required for growth of bacteria is included at a high concentration in a synthetic or natural polymeric substance within a binding-type carrier. And, a porous material (a synthetic or natural inorganic porous material) in which microorganisms can easily live are mounted therearound to increase the living density of microorganisms.

Such a carrier is installed in a reactor. Around the carrier, a liquid to be treated is contacted with a group of microorganisms in a stream parallel or perpendicular to the carrier to degrade a substrate.

Consequently, the decrease in activity of bacteria due to toxicity of a polymeric material to be used is prevented. Further, inhibition of growth is prevented by adjusting a thickness of a polymer product or an inorganic porous material

constituting a carrier and a pore of the inorganic porous material. Still further, physiological peeling of bacteria is prevented according to types and characteristics of bacteria and fluidity of a reactor to be used.

Moreover, the invention is also based on the finding in studies on culture of methane bacteria at high concentrations, that is, a density of bacteria can be increased in view of the knowledge that the growth of bacteria is controlled by deficiency of a trace element or an inorganic nutrient salt required for growth of bacteria.

In addition, this finding agrees with the minimum law of Liebig that when only one of substances required for bacteria is deficient, growth of any bacteria is stopped. From this finding, in a method for feeding substances required for growth of various bacteria acting in a bioreactor or a wastewater disposal apparatus, these substances are included at high concentrations inside a carrier, and moved from the inside to the surface of the carrier by diffusion to supply the same to bacteria living on the surface thereof. Bacteria digest the substances to continue the growth, whereby the high density of bacteria can be maintained. This has been also identified by experiments.

With respect to the polymeric material of the invention including a trace element or an inorganic nutrient salt, typical examples of a synthetic polymeric material are polymers known

as water-absorbable polymers, such as an acrylic polymer, a methacrylic polymer, a vinyl alcohol polymer, a vinyl ester polymer, a polyether, a polyester and a polyolefin polymer, copolymers thereof, and gels thereof. A trace element or an inorganic nutrient salt is included and immobilized in these polymeric materials. The polymeric material may be formed as an agglomerate of a finely divided polymer.

Further, a polymeric material may be a natural polymeric material. It may include various materials such as an agar gel, celluloses and polysaccharides.

The inorganic porous material of the invention may include various synthetic or natural porous materials. Ceramics, porous concretes and volcanic porous materials such as rock wool and pumice are available. When the porosity is slightly less than 95 % or 95 to 98 % including an approximate range slightly exceeding 98 %, preferably 95 to 98 % and a diameter of the pore is 2 to 0.1 mm, the porous material is most suitable for growth of bacteria. Further, it is required to select a porous material which does not elute a substance (heavy metal such as Cd) harmful for growth of bacteria used. Moreover, it is important to select a natural porous material which is not easily degraded with anaerobic bacteria.

The element polymer product including the trace element and the inorganic nutrient salt is, in the carrier of the invention, laminated by being held with the inorganic porous

material. The whole carrier may take various shapes. Examples thereof can include a cylindrical carrier, a rectangular carrier, a flat-plate-like carrier and a curved-plate-like carrier.

The trace element and the inorganic nutrient salt in the invention may be selected from trace metal elements and inorganic nutrient salts ordinarily considered. Examples of the trace element include Mg, Mn, Fe, Ni, Co, Cu, Se, Mo, Al, W, Ca and B. Examples of the inorganic nutrient salt include a phosphate and a carbonate of an alkali metal.

Bacteria themselves are bound and immobilized, for example, on the surface, in the inner pores or in the voids of the porous material through covalent bond, physical adsorption or ionic bond. Bacteria may previously be included in the carrier, laminated on the surface of the carrier, or both included therein and laminated thereon, depending on characteristics of the carrier.

The drawings appended illustrates the carrier of the invention. Fig. 1 shows a cylindrical carrier 1. A polymer product 11 as a core includes a trace element and an inorganic nutrient salt for growth of bacteria. This core has a structure that it is laminated by being held with a surrounding inorganic porous material 12.

Fig. 2 shows a multiple cylindrical carrier 1. In this case, a cylinder having a hollow portion 13 has a structure that a polymer product 11 including a trace element and a nutrient

salt for growth of bacteria is laminated by being held with the inorganic porous materials 12.

Fig. 3 shows a flat-plate-like carrier 1. This carrier has a structure that the flat-plate-like polymer product 11 is laminated by being held with the inorganic porous materials 12.

Fig. 4 shows a carrier 1 of a honeycomb structure. On a wall surface surrounding a hollow portion 13, the polymer product 11 as above described is laminated by being held with the inorganic porous materials 12.

Such a carrier is installed in, for example, a bioreactor. In the application to the bioreactor, the carrier and its arrangement can be determined in consideration of a purpose of culture, types and properties of bacteria, a substrate and a diffusion rate of a trace element and an inorganic nutrient salt to conduct the culture most efficiently in relation to a liquid flow in the bioreactor.

For example, Fig. 5 shows an example in which the carrier 1 in Figs. 1 to 3 is installed in the reactor 2, and Figs. 6A and 6B show examples in which plural holes are formed approximately in parallel in the rectangular and cylindrical inorganic porous materials 12 and cylindrical and particulate polymer products 11 including trace elements and inorganic nutrient salts are packed in the holes. In Fig. 7, for example, the flat-plate-like carriers 1 in Fig. 3 are mounted within

the reactor 2 in zigzag state vertically and horizontally to control the flow as shown in a side sectional view of Fig. 7A and a plan view of Fig. 7B. Fig. 8 shows an example in which the carriers 1 in Figs. 1 to 3 are installed such that the side portion of the inorganic porous material 2 is approximately parallel to a flow direction of a culture solution. Fig. 9 shows a structure in which the carriers 1 in Fig. 3, Fig. 4 and Fig. 6A are laminated in a bioreactor. In this case, the side surface is approximately parallel to the flow. In the embodiment of Fig. 9, it is identified that an excellent performance is exhibited by making a packing rate of the polymer products 15 to 25 % (volume percentage) of an effective volume.

When the carrier of the invention is used, for example, 10 to 25 g dry cells/liter can be expected as a cell concentration. In the ordinary method, it was approximately 1 to 5 g dry cells/liter.

Further, when an amount of a substrate in a degradation system in wastewater disposal is represented by S, the degradation thereof is represented by the formula:

$$\left(\frac{dS}{dt}\right) = -\mu \cdot X / Y_{x/s}$$

wherein μ represents a specific growth rate of bacteria, X represents a density of bacteria, and $Y_{x/s}$ represents a yield of bacteria (which is a fixed value depending on bacteria).

In the invention, the reactor is operable when μ is close

to μ_{\max} , whereby the density x of bacteria can be increased by 2 to 10 times in comparison with that in an ordinary reactor. Accordingly, a degradation rate of a suspension culture apparatus is increased by 2 to 10 times in comparison with that in an ordinary apparatus. It can be increased by several times in comparison with that in a bioreactor using an ordinary carrier.

The invention is now described in more detail by referring to the following Example.

EXAMPLE

A trace element and a metal salt as an inorganic nutrient salt were supported on a PVA (polyvinyl alcohol) polymer gel obtained by dissolving approximately 16 % by weight of a polymeric material having a weight average molecular weight of approximately 2,000 and a saponification degree of 98 % in water and crosslinking the solution with saturated boric acid. The resulting polymer product was filled in six holes of rock wool as an inorganic porous material so as to provide a cylindrical shape shown in Fig. 6B. Methane bacteria were incubated such that the volume of the polymeric material filled in the carrier corresponded to 20 % of a liquid portion. This was compared with a case in which a carrier was not used. The liquid temperature was set at 5°C, 15°C and 25°C.

Table 1 shows a trace metal element, Table 2 a basic

inorganic salt, and Table 3 a composition of a vitamin solution respectively.

Further, Table 4 shows physical properties and a composition of rock wool as an inorganic porous material.

Table 1

Component	Concentration ($\mu\text{g/liter}$)
$\text{MgCl} \cdot 6\text{H}_2\text{O}$	410
$\text{MnCl} \cdot 4\text{H}_2\text{O}$	50
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	50
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	12
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	10
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	10
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10
Na_2SeO_3	8
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	2.4
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1
$\text{AlK}(\text{SO}_4)_2$	1
H_3BO_3	1.8
$\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$	1

Table 2

Component	Concentration (mg/liter)
KH_2PO_4	3400
K_2HPO_4	3400
NH_4Cl	2130
Na_2CO_3	2540
Resazurin	2

Table 3

Component	Concentration ($\mu\text{g/liter}$)
Biotin	20
Folic acid	20
Pyridoxine hydrochloride	20
Thiamine hydrochloride	100
Riboflavin	50
Nicotinic acid	50
Calcium DL-pantothenate	50
p-Aminobenzoic acid	50
Lipoic acid	50

Table 4

Physical properties and composition of rock wool

Density (kg/m^3)	80 \pm 12
True specific gravity	2.9
Porosity (%)	95 - 98
Composition (%)	SiO ₂ : 42, Al ₂ O ₃ : 15, CaO: 33, MgO:6, Fe:0.5, TiO ₂ :0.9, MnO: 0.2, Na ₂ O:1, K ₂ O:0.8

The incubation of methane bacteria at various incubation temperatures was compared with that in the absence of a carrier. As a result, with respect to amounts of methane bacteria and a methane production rate, the results shown in Table 5 were obtained.

Table 5

Temperature ($^{\circ}\text{C}$)	Amounts of methane bacteria	Methane production rate
25	4.8 times	5.8 times
15	2.5 times	5.1 times
5	2.3 times	4.5 times

In Table 5, concentrations of a trace element and an

inorganic nutrient salt in a carrier were 1,000 times as high as those in an ordinary method. It has been identified that with respect to the polymer product and the inorganic porous material charged into a reactor, it is mainly the volume of the polymer product and the porosity of the inorganic porous material that influence the incubation. However, when a porosity is 95 to 98 %, an apparent volume of the porous material is almost unchanged.

When the carrier takes the shape in Fig. 5, it is estimated that the upper limit of the volume of the polymer product is approximately 25 % of a liquid volume.

As is clear from Table 5, compared with the absence of the carrier, the presence of the carrier provides the excellent effects that the amounts of methane bacteria are 2.3 to 4.8 times and the methane production rate is 4.5 to 5.8 times. Consequently, it is considered that the same excellent results can be obtained in a model plug flow-type bioreactor shown in Figs. 7 to 9 as well. Therefore, as an application to an actual biosystem, a test was conducted with nitrifying and denitrifying bacteria such that a carrier of Fig. 6A provided by incorporating the polymer products in a porous concrete block was mounted on a bottom of a sewage treatment channel. As a result, removal rates of 50 to 60 % and 40 to 50 % (annual average) could be attained in T-N and T-P, respectively.

As has been thus far described in detail, the invention

What is claimed is:

1. A bacteria growth carrier including a trace element characterized that an element polymer product obtained by including a trace element or the trace element and an inorganic nutrient salt for growth of bacteria in a synthetic or natural polymeric material is laminated by being held with a synthetic or natural inorganic porous material.
2. The bacteria growth carrier as claimed in claim 1, which is a particulate, cylindrical or plate-like carrier.
3. The bacteria growth carrier as claimed in claim 1, which has a honeycomb structure.

ABSTRACT

Provided is a bacteria culture carrier in which a polymer product having a trace element and an inorganic nutrient salt for growth of bacteria included therein is laminated by being held with an inorganic porous material. This carrier is useful in a bioreactor or wastewater disposal with a high activity and a high density of bacteria.

Fig.1

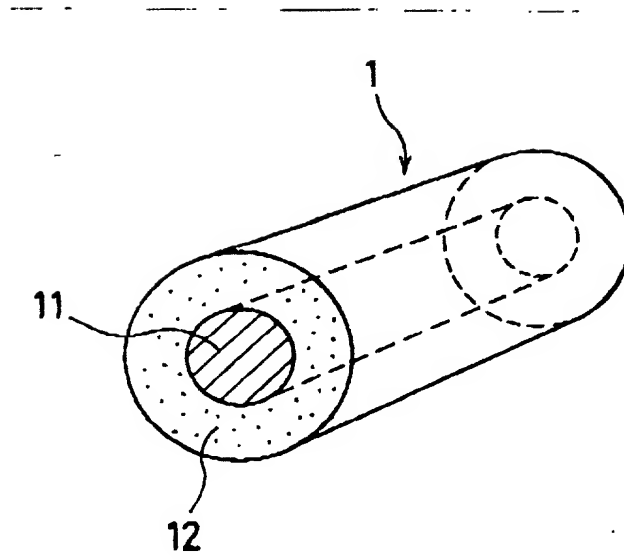


Fig.2

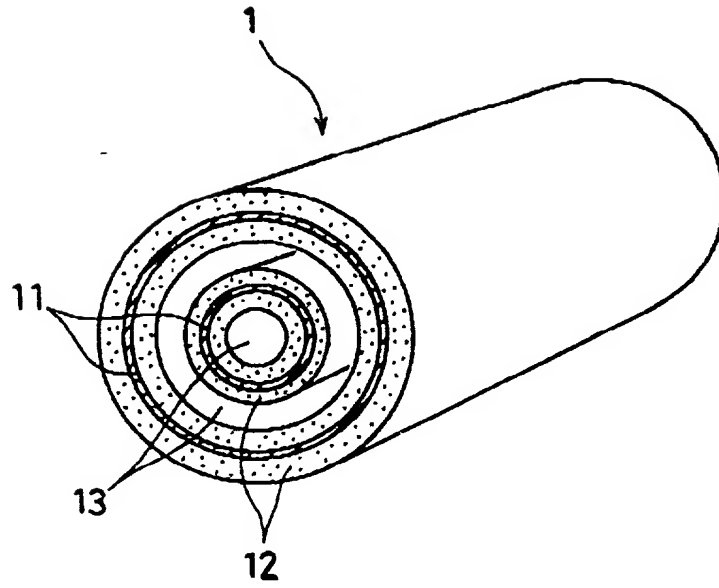


Fig.3

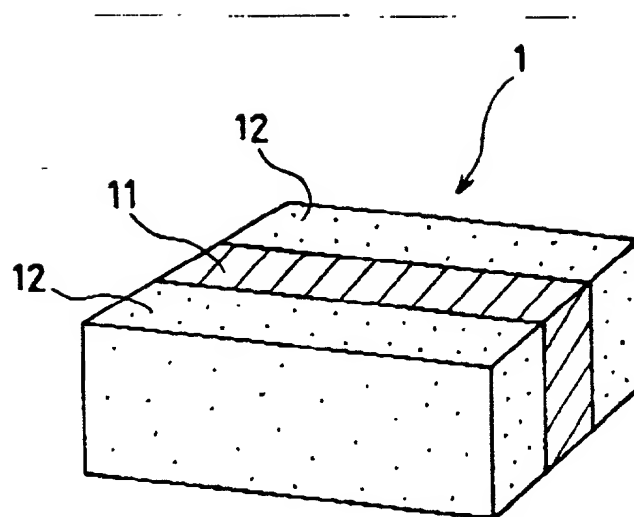


Fig.4

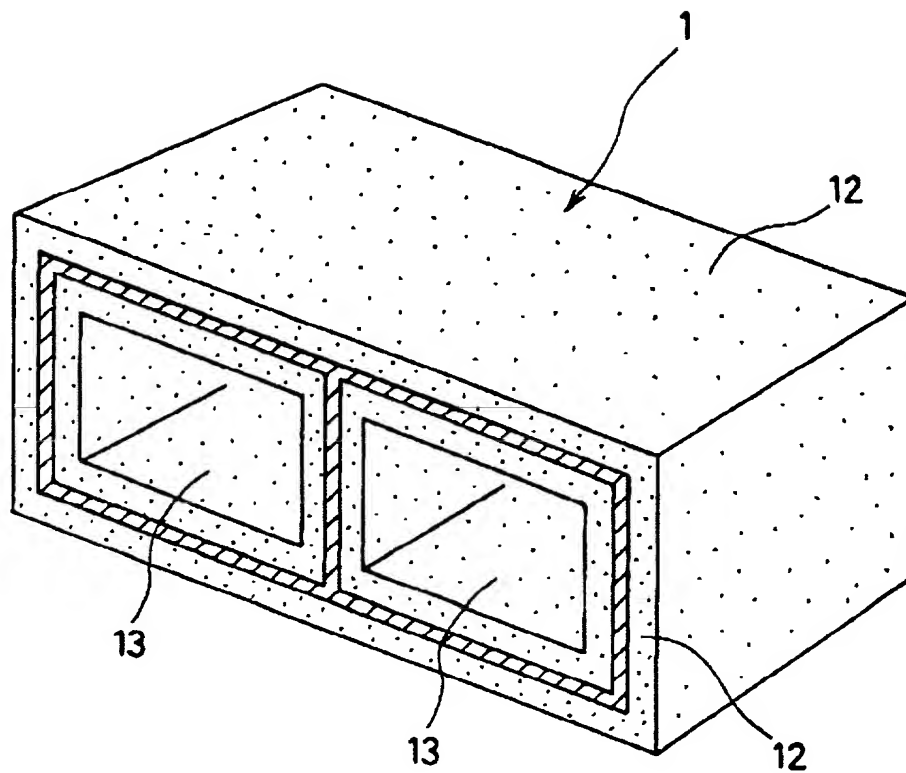


Fig.5

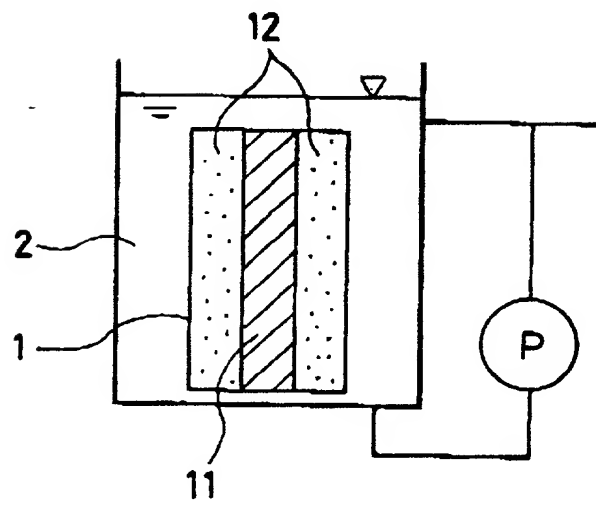


Fig.6

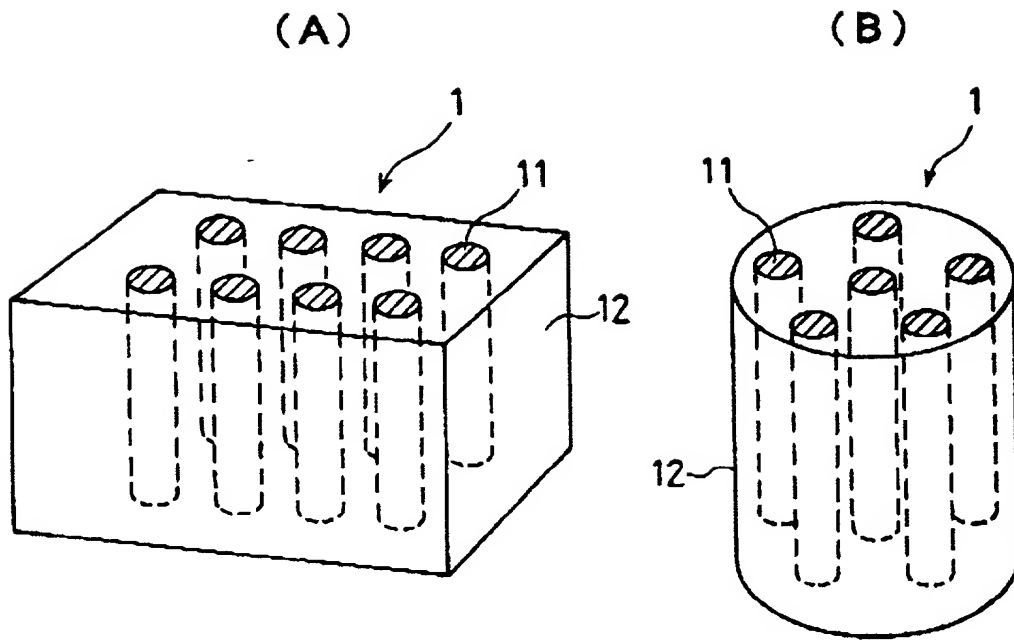
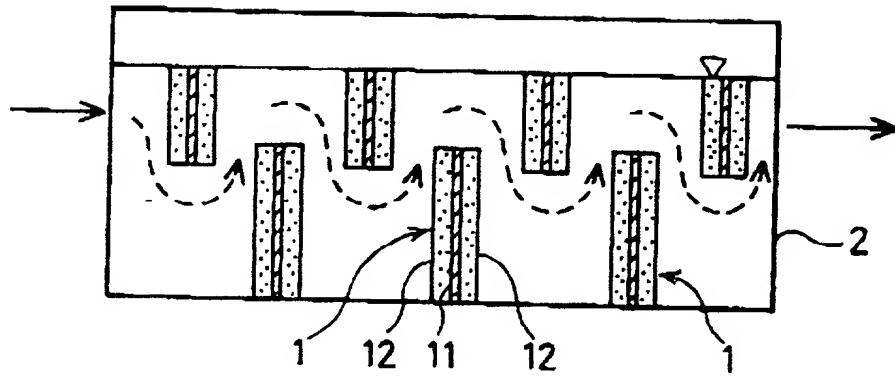


Fig.7

(A)



(B)

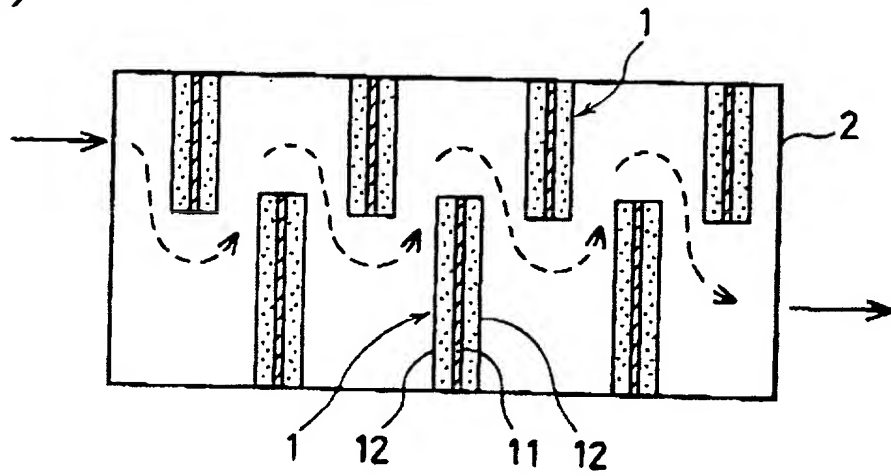


Fig.8

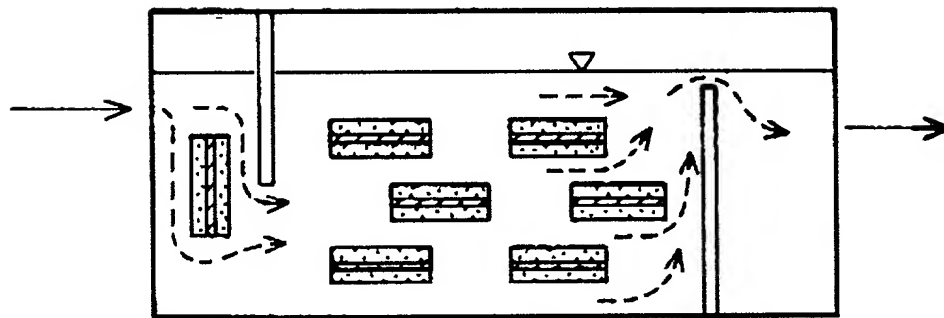
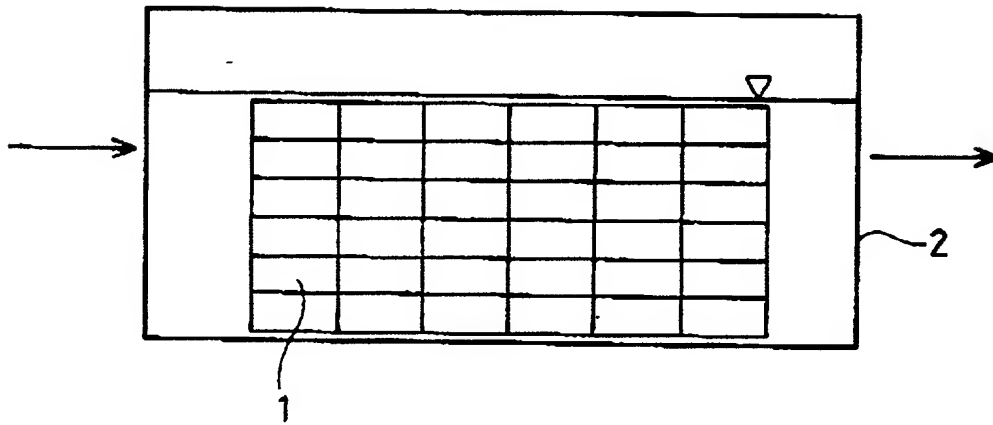


Fig.9



DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATION

☐ Original ☐ Supplemental ☐ Substitute ☒ PCT ☐ DESIGN

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Title: BACTERIA GROWTH CARRIER INCLUDING TRACE ELEMENT

of which is described and claimed in:

☐ the attached specification, or
☐ the specification in application Serial No. _____, filed _____, and with amendments through _____, or
☒ the specification in International Application No. PCT/JP99/05758, filed October 19, 1999, and as amended on _____ (if applicable).

I hereby state that I have reviewed and understand the content of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge my duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim priority benefits under Title 35, United States Code, §119 (and §172 if this application is for a Design) of any application(s) for patent or inventor's certificate listed below and have also identified below any application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NO.	DATE OF FILING	PRIORITY CLAIMED

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	U.S. FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

And I hereby appoint Michael R. Davis, Reg. No. 25,134; Matthew M. Jacob, Reg. No. 25,154; Warren M. Cheek, Jr., Reg. No. 33,367; Nils Pedersen, Reg. No. 33,145; Charles R. Watts, Reg. No. 33,142; and Michael S. Huppert, Reg. No. 40,268, who together constitute the firm of WENDEROTH, LIND & PONACK, L.L.P., as well as any other attorneys and agents associated with Customer No. 000513, to prosecute this application and to transact all business in the U.S. Patent and Trademark Office connected therewith.

I hereby authorize the U.S. attorneys and agents named herein to accept and follow instructions from NISHIZAWA & ASSOCIATES as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and myself. In the event of a change in the persons from whom instructions may be taken, the U.S. attorneys named herein will be so notified by me.

Direct Correspondence to Customer No:



000513

PATENT TRADEMARK OFFICE

Direct Telephone Calls to:

WENDEROTH, LIND & PONACK, L.L.P.
2033 "K" Street, N.W., Suite 800
Washington, D C 20006-1021

Phone (202) 721-8200
Fax.(202) 721-8250

Full Name of First Inventor	FAMILY NAME MAEKAWA	FIRST GIVEN NAME Takaaki	SECOND GIVEN NAME
Residence & Citizenship	CITY Tsukuba-shi, Ibaraki	STATE OR COUNTRY Japan	COUNTRY OF CITIZENSHIP Japan
Post Office Address	ADDRESS 1-1-1, Tennodai, Tsukuba-shi, Ibaraki	CITY Japan	STATE OR COUNTRY Japan

Full Name of Second Inventor	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
Residence & Citizenship	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
Post Office Address	ADDRESS	CITY	STATE OR COUNTRY ZIP CODE

Full Name of Third Inventor	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
Residence & Citizenship	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
Post Office Address	ADDRESS	CITY	STATE OR COUNTRY ZIP CODE

Full Name of Fourth Inventor	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
Residence & Citizenship	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
Post Office Address	ADDRESS	CITY	STATE OR COUNTRY ZIP CODE

Full Name of Fifth Inventor	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
Residence & Citizenship	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
Post Office Address	ADDRESS	CITY	STATE OR COUNTRY ZIP CODE

Full Name of Sixth Inventor	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
Residence & Citizenship	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
Post Office Address	ADDRESS	CITY	STATE OR COUNTRY ZIP CODE

I further declare that all statements made herein of my own knowledge are true, and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1st Inventor Takaaki Maekawa Date March 27, 2002
Takaaki MAEKAWA
2nd Inventor _____ Date _____
3rd Inventor _____ Date _____
4th Inventor _____ Date _____
5th Inventor _____ Date _____
6th Inventor _____ Date _____

The above application may be more particularly identified as follows:

U.S. Application Serial No. _____ Filing Date February 28, 2002

Applicant Reference Number 99-F-042US/NT Atty Docket No. 2002-0279A

Title of Invention BACTERIA GROWTH CARRIER INCLUDING TRACE ELEMENT